Nerve Growth Factor and Prostaglandins in the Urine of Female Patients With Overactive Bladder

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Purpose: NGF and PGs in the bladder can be affected by pathological changes in the bladder and these changes can be detected in urine. We investigated changes in urinary NGF and PGs in women with OAB.

Materials and Methods: The study groups included 65 women with OAB and 20 without bladder symptoms who served as controls. Evaluation included patient history, urinalysis, a voiding diary and urodynamic studies. Urine samples were collected. NGF, PGE_2, $PGF_{2\alpha}$ and PGI_2 were measured using enzyme-linked immunosorbent assay and compared between the groups. In addition, correlations between urinary NGF and PG, and urodynamic parameters in patients with OAB were examined.

Results: Urinary NGF, PGE_2 and $PGF_{2\alpha}$ were significantly increased in patients with OAB compared with controls ($p < 0.05$). However, urinary PGI_2 was not different between controls and patients with OAB. In patients with OAB urinary PGE_2 positively correlated with volume at first desire to void and maximum cystometric capacity ($p < 0.05$). Urinary NGF, $PGF_{2\alpha}$ and PGI_2 did not correlate with urodynamic parameters in patients with OAB.

Conclusions: NGF and PGs have important roles in the development of OAB symptoms in female patients. Urinary levels of these factors may be used as markers to evaluate OAB symptoms.

Key Words: urinary incontinence, bladder, nerve growth factor, prostaglandins, female

OAB is defined by the International Continence Society as urinary urgency with or without urge incontinence, usually with urinary frequency and nocturia, in the absence of pathological or metabolic factors that would explain these symptoms. Recent epidemiological studies have shown that the overall prevalence of OAB in women is 16.9%.[1] There have been many studies of OAB but the causes and mechanisms of this malady remain poorly understood. OAB symptoms can be affected by neurotransmitter release or inflammatory mediators. In particular these symptoms can be attributable to NGF and PGs.

NGF is a secretory protein that has a critical role in the development of the peripheral nervous system. Increased NGF expression in the bladder may contribute to storage symptoms, such as urgency and frequency, in patients with OAB.[2,3] It has also been reported that PGs have an important role in regulating lower urinary tract function.[4] PGs are locally synthesized in the bladder muscle and mucosa. This synthesis is initiated by detrusor muscle stretch, bladder nerve stimulation, bladder mucosa damage and inflammation mediators. PGs are involved in the micturition reflex by decreasing thresholds of the stimuli necessary to trigger bladder contraction through activation of the capsaicin sensitive afferent nerves.[5–7] Therefore, NGF and PGs can be related to bladder storage symptoms in patients with OAB. It is assumed that alterations in the levels of these chemical transmitters can eventually be detected in the urine. We investigated alterations in urinary NGF and PG in women with OAB symptoms with the aim of identifying the possible diagnostic value of these substances in the evaluation of women with OAB.

MATERIALS AND METHODS

Patients and Evaluation

A total of 65 women with a mean age of 55.5 years (range 21 to 78) with OAB who had symptoms such as urgency, frequency and/or urge incontinence were evaluated. Patients who had a frequency of greater than 8 times daily based on voiding diaries were enrolled in this study. Subjects with OAB who were admitted to this study did not have any neurological disease and urinalysis revealed no abnormalities. The control group included 20 healthy female volunteers with a mean age of 47.5 years (range 28 to 65) who had no bladder symptoms and no abnormality on urinalysis.

Patient voiding diaries for 3 consecutive days were evaluated and urodynamic studies were performed in all subjects with OAB. Urodynamic parameters were assessed, including the maximum flow rate, post-void residual volume, volume at first desire to void, maximum cystometric capacity and detrusor overactivity. Detrusor overactivity was defined as any involuntary detrusor muscle contraction more than 10 cm H_2O during the filling phase that was seen spontaneously or associated with the sensation of urgency.

Urine Collection and Preparation

After receiving permission from all subjects voided urine was collected when subjects felt a full sensation. Urine samples were centrifuged at 5,000 × gravity for 10 minutes. The

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supernatant was separated into aliquots in 1.5 ml tubes and preserved in a refrigerator at −20C.

Estimation of Urinary NGF and PGs

Urinary NGF, PGE_2, $PGF_{2\alpha}$ and PGI_2 were measured using ELISA. Urinary NGF was determined using the Emax® ImmunoAssay System, which has a detection limit of 5 pg/ml. To coat 96-well plates with antibody 100 μl polyclonal anti-NGF antibody diluted in carbonate coating buffer, pH 9.7, were pipetted into each well and incubated overnight at 4C. The wells were washed once with tris buffered saline-Tween washing buffer. To prevent any nonspecific reaction they were incubated with 200 μl 1 × block and sample buffer for 1 hour at room temperature. NGF standards or 100 μl urine were then added to the wells. After 6 hours the wells were washed 5 times, 100 μl secondary antibody (2.5 μl monoclonal anti-NGF antibody diluted in 10 ml 1 × block and sample buffer) were added to each well and the plates were incubated overnight at 4C. After washing 5 times 100 μl anti-rat IgG horseradish peroxidase were added to each well and incubated for 2½ hours at room temperature. The wells were again washed 5 times and then incubated with 100 μl TMB (3,3′5,5′ tetramethyl benzydine) substrate solution for 10 minutes at room temperature. Hydrochloric acid (1 N 100 μl) was added to terminate the reactions.

The amount of NGF was determined following absorbance measurement with a SpectraMax® 250 ELISA Reader. To measure the amounts of PGE_2, $PGF_{2\alpha}$ and PGI_2 a High Sensitivity ELISA kit (R and D Systems, Minneapolis, Minnesota) was used. In antibody coated wells TA, NSB, maximum binding and substrate blank wells were marked for comparison. Assay buffer (a buffered protein base) was added to the zero standard (B_0) (100 μl) and NSB (150 μl) wells. PG standards and 100 μl diluted urine samples were pipetted into the remaining wells. High sensitivity conjugate (50 μl) was added to each well except the TA and substrate blank wells. High sensitivity antibody solution (50 μl) was dispensed into all wells except the NSB, TA and substrate blank wells. The wells were covered with an adhesive strip and allowed to react. After washing 3 times with 200 μl washing buffer all liquid was removed from the wells. Conjugate (5 μl) was added to the TA well and 200 μl p-nitrophenyl phosphate were added to each well. Stop solution (50 μl trisodium phosphate solution) was pipetted into each well and the PG concentration was determined following absorbance measurement with the SpectraMax® 250 ELISA Reader.

Urinary NGF and PGs were compared in controls and patients with OAB. These urinary levels were correlated with urodynamic parameters, namely the maximum flow rate, post-void residual urine, volume at first desire to void, maximum cystometric capacity and detrusor overactivity, in patients with OAB.

Urinary NGF and PGs in controls and female patients with OAB

	Mean Control ± SEM	Mean OAB ± SEM
NGF (ng/ml)	2.45 ± 0.58	30.92 ± 5.23*
PGE_2 (ng/ml)	0.45 ± 0.15	1.54 ± 0.15*
$PGF_{2\alpha}$ (ng/ml)	0.28 ± 0.10	0.94 ± 0.17*
PGI_2 (ng/ml)	0.42 ± 0.09	0.52 ± 0.07

* $p < 0.05$.

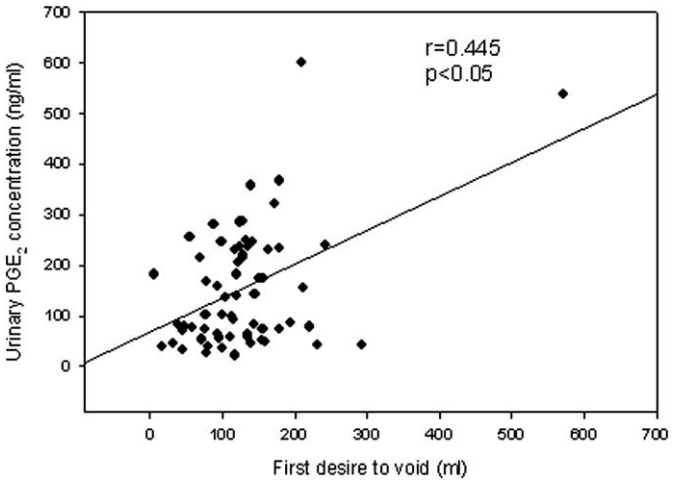

FIG. 1. Urinary PGE_2 positively correlated with volume at first desire to void in women with OAB.

Statistics

Results are expressed as the mean ± SEM. SigmaStat® for Windows® was used for statistical analysis. Student's t test was used to test for statistical significance between controls and subjects with OAB. Pearson's product moment correlation was used to determine correlations between NGF and PG, and urodynamic parameters with $p < 0.05$ considered statistically significant.

RESULTS

Symptom and Urodynamic Studies

All subjects with OAB had urgency, while urge incontinence was noted in 21 (32%). On urodynamic studies volume at first desire to void and maximum cystometric capacity were 129.2 ± 78.4 and 291.1 ± 114.3 ml, respectively. Detrusor overactivity was detected in 23 subjects with OAB (35.0%).

Urinary NGF and PGs

Urinary NGF was significantly increased in subjects with OAB compared with controls ($p < 0.05$). In addition, urinary PGE_2 and $PGF_{2\alpha}$ were significantly increased in subjects with OAB compared with controls ($p < 0.05$). However, urinary PGI_2 was not different between controls and subjects with OAB (see table). In subjects with OAB urinary PGE_2 correlated positively with volume at first desire to void and maximum cystometric capacity ($p < 0.05$, figs. 1 and 2). However, urinary PGE_2 did not correlate with the maximum flow rate, post-void residual volume or detrusor overactivity. Urinary NGF, $PGF_{2\alpha}$ and PGI_2 did not correlate with urodynamic parameters in subjects with OAB.

DISCUSSION

The results of this study show that urinary NGF and PGs are associated with OAB symptoms. Although the pathophysiology of OAB is not fully understood, OAB symptoms are partially caused by changes in afferent nerves, and NGF and PG have important roles in this process.